

THE RESISTANCE OF IMMATURE ERYTHROCYTES TO HEAT

Raphael Isaacs, ... , Benjamin Brock, George R. Minot

J Clin Invest. 1925;1(5):425-433. <https://doi.org/10.1172/JCI100022>.

Research Article

Find the latest version:

<https://jci.me/100022/pdf>



THE RESISTANCE OF IMMATURE ERYTHROCYTES TO HEAT*

BY RAPHAEL ISAACS†, BENJAMIN BROCK AND GEORGE R. MINOT

(From the Medical Service of the Collis P. Huntington Memorial Hospital of Harvard University)

(Received for publication, December 26, 1924)

INTRODUCTION

Knowledge concerning the differences between mature cells and those in the process of development adds to the understanding of the vital problem of growth. The red blood corpuscles offer excellent material for the study of adult and immature forms, because of the recognition of four successive stages in their development. These include the nucleated red cells, the reticulated forms, the granule red cells (cells described by Isaacs, with single, refractile, non-staining granules) and finally the mature erythrocytes. Heat as a "cytotoxic" agent does not appear to have been utilized, heretofore, for the purpose of studying different kinds of erythrocytes.

The destructive and alterative action of heat on erythrocytes was described some sixty years ago by Klebs, Rollett, Beale and Schultze. Since then there appear but few papers on this subject; among them are those quoted by Krehl and Marchand. No quantitative data, however, have been found, nor any observations on the behavior to heat of erythrocytes in different diseases. The present study was undertaken to discover whether the red cells in various diseases reacted differently towards heat, and to learn if there was any quantitative relationship between the ages of red cells and their resistance to heat.

* This paper is No. 40 of a series of studies in metabolism from the Harvard Medical School and allied hospitals. The expenses of this investigation have been defrayed in part by a grant from the Proctor Fund of the Harvard Medical School, for the Study of Chronic Disease, and in part by the Edward Hickling Bradford Fellowship.

† Bradford Fellowship.

METHODS AND MATERIAL

The following procedures were utilized in studying the effect of heat on the red blood corpuscles of patients and normal persons.

Blood from an arm vein was mixed with sufficient crystalline sodium citrate to make a 0.2 per cent solution. Blood films, supravitaly stained with brilliant cresyl blue and counterstained with Wright's stain were made on cover glasses. Such preparations were used to study the condition of the blood before and after it had been subjected to heat. One cubic centimeter of blood was heated in small test tubes, suspended in a water bath at a temperature ranging from 55° to 58°C. Whenever a pathological blood was tested, blood from a healthy person, as a control, was treated at the same time in an identical manner. Every care was taken to prevent further alteration of the blood by such physical means as mixing, shaking or foam formation.

Capillary glass tubes, washed free of alkali were used in other experiments and blood was taken directly into them from a skin puncture, and, as it ran from the wound, was mixed with a few crystals of sodium citrate. Then the tubes were sealed and heated in a water bath. There was no appreciable difference in the results obtained from these two methods.

In addition to blood from five healthy individuals, used as controls with each heating, blood from 31 patients (28 with anemia and 3 of erythremia with polycythemia) were studied. All 31 of the patients showed a percentage of immature red cells above normal. The 28 patients with anemia included 8 cases of chronic myelogenous and 3 of chronic lymphatic leukemia, 6 of Hodgkin's disease, 3 of pernicious anemia in relapse, 2 of chronic thrombopenic purpura, 4 of familial (one splenectomized) and 1 of acquired, chronic hemolytic jaundice and 1 of hemolytic anemia of pregnancy.

EFFECT OF HEATING NORMAL WHOLE BLOOD

The red cells in normal blood subjected to heat up to 50°C. for 30 minutes show little or no visible change. At 65°C. and higher they are destroyed almost completely. Normal blood when heated to 55°C. for 30 minutes, shows a marked alteration in the shape and

appearance of the red cells (fig. 1). Seventy to 80 per cent of the cells are broken up, or appear as mere shadows, or present unusual

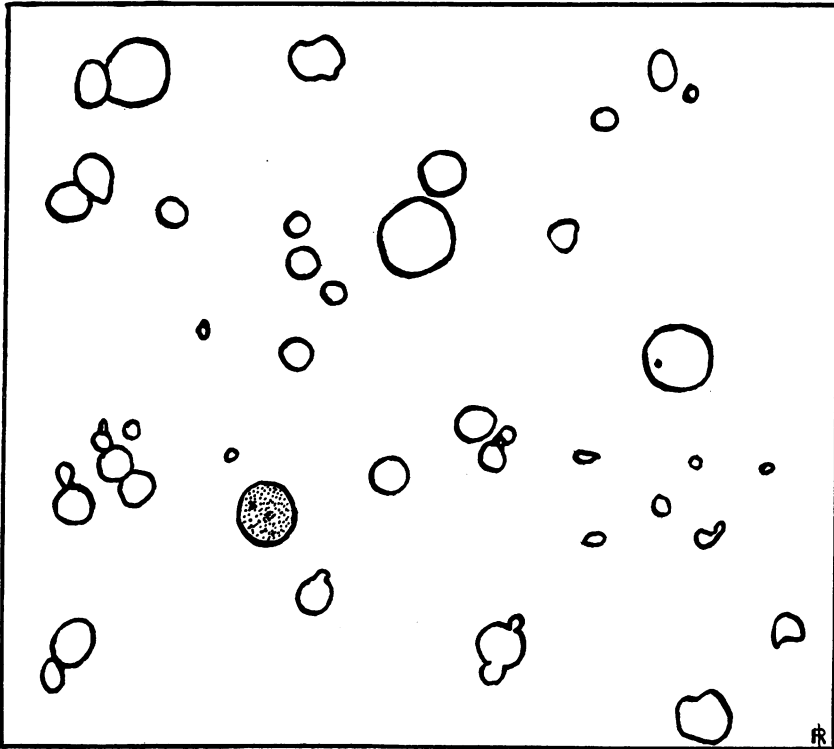


FIG. 1. REPRESENTATIVE FIELD OF A COVERGLASS FILM OF NORMAL BLOOD WHICH PREVIOUSLY HAD BEEN HEATED TO 55°C. FOR 30 MINUTES

A granule red cell and a reticulated red cell (basophilic) are shown. Fragmentation is marked, and but few of the cells are intact. The stroma of the hemolyzed red corpuscles is not shown in the drawing. It is visible in the stained preparations and the "shadows" fill the spaces between the intact cells. This figure and figure 2 represent microscope fields in which the total number of intact cells plus "shadows" is approximately equal.

Camera lucida drawing (table level). Leitz ocular 8, oil immersion objective 2 mm.

and bizarre forms. Spherical bodies resembling microcytes are a feature, and smaller irregular fragments and filaments of cells are numerous. Many larger cells, less affected, are deformed and elon-

gated. A few cells are apparently little altered, but practically all lose their normal staining characteristics. The cells stain uniformly, and homogeneously, and perhaps are somewhat more basophilic than normal. The reticulum of the reticulocytes, as Key observed, does not stain so well as in unheated specimens. The microcyte formation is present before films are made and is not produced by trauma.

The cells which broke up in greatest numbers were the mature cells, lacking the structures (reticulum, granules) that characterize young cells while the immature cells remained intact. Table 1 shows the effect of heating a sample of normal blood. Among the intact cells the percentage of immature ones is strikingly increased. The absolute number of young cells before and after heating was approximately the same (within experimental error) so that it is not likely that heating produced forms resembling the different types of immature

TABLE 1
Effects of heating specimen of normal blood to 55°C. for thirty minutes
(Numbers represent the percentage of intact red cells)

	Before heating	After heating
Reticulated and basophilic red cells	1.4	15.1
Granule cells	1.8	26.3
Total recognizable young red cells	3.2	41.4

cells. Similar results were obtained repeatedly, though the actual number of intact cells varied. In blood remaining overnight at 4°C. or at 37°C. all red cells, especially reticulocytes, became more resistant to heat than fresh corpuscles. These observations lead to the conclusion that *normal immature red blood corpuscles are more resistant to heat than adult corpuscles.*

EFFECT OF HEATING PATHOLOGICAL BLOODS

The observation that immature red cells, including blasts, are more resistant to heat than adult red cells was brought out much more strikingly in studying the 31 pathological bloods containing an increased (often high) percentage of reticulocytes. Scrutiny of many preparations showed that reticulated microcytes, macrocytes and normocytes resisted the action of the heat in the same manner.

Heat appeared to break up reticulated megalocytes in two cases of pernicious anemia. Actual measurements of cells indicated that red cells shrink during heating, but this contraction alone does not seem to account for the disappearance of the extremely large corpuscles. Thus the actual size of the cell, unless a megalocyte, plays no rôle in its resistance to heat. *The only differences produced by heating the blood from the patients with the various diseases were quantitative (proportional to the degree of immaturity) and no specific qualitative differential criteria were noted.* In some bloods containing a high percentage of recognizable immature red cells, there remained, after heating, a relatively large number, compared to normal, of cells not containing nuclei, reticulum or granules. This was particularly true of the blood from cases of chronic hemolytic jaundice.

The blood from four untreated cases of chronic hemolytic jaundice showed a greatly increased fragility to hypotonic salt solution of the red blood corpuscles and a distinct increase of the reticulocytes; both of which features are characteristic of this condition. Owing to the decreased osmotic resistance of the red cells one might suppose that their destruction by heat would occur more readily than normal. Such is not, however, the case. In chronic hemolytic jaundice the response of the red cells to heat confirms the view that the immature cells are more resistant than the mature cells to this physical agent. The data recorded in table 2, illustrate the effect of heat on the red cells of this condition as contrasted with the normal. As the table shows, the bulk of the red cells resist destruction. The number of intact cells per microscope field (oil immersion) in the control (A) blood film after heating was, on the average, for 50 fields, 9.9, while in comparable films of the hemolytic jaundice blood (A), the average number of intact cells was 238 (fig. 2). In spite of the differences in figures for such data as shown in table 2 obtained by carefully observing and counting both intact and broken cells for controls and cases, the resistance to heat of the bulk of the red cells in chronic hemolytic jaundice is distinct enough perhaps to aid in diagnosis.

Red cells from the blood of normal persons and of patients with chronic hemolytic jaundice were mixed with 0.7 and 0.85 per cent sodium chloride solution and studied before and after heating. Here another factor than heat influences the results. The red cells of a

particular patient's blood were not hemolyzed before heating by 0.7 per cent sodium chloride solution, but were hemolyzed in small numbers by 0.6 per cent salt solution. Normal cells were similarly affected by 0.42 per cent salt solution. After heating, this patient's cells mixed with 0.7 per cent sodium chloride solution practically all

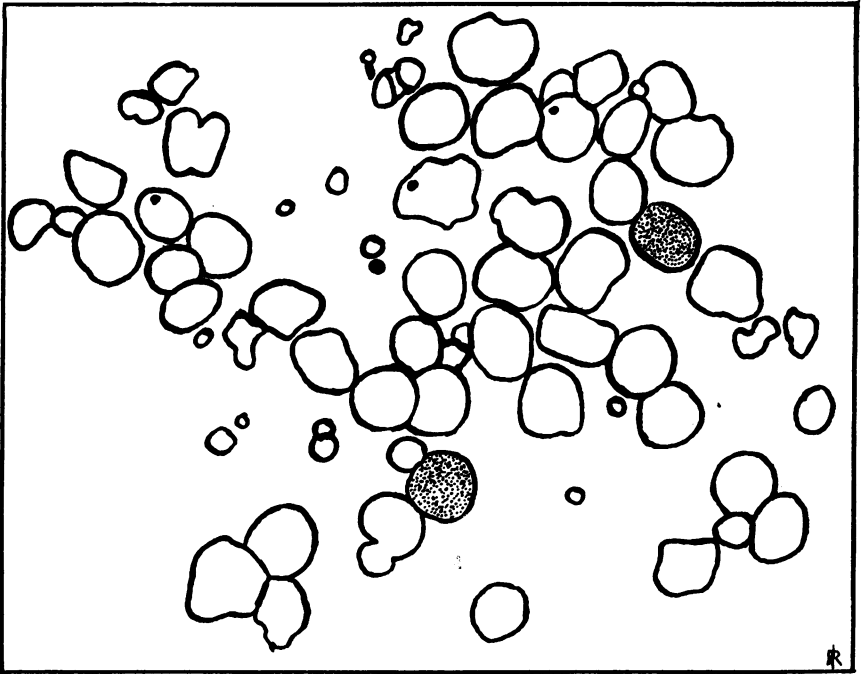


FIG. 2. REPRESENTATIVE FIELD OF A COVERGLASS FILM OF BLOOD AFTER HEATING TO 55°C. FOR 30 MINUTES, FROM A PATIENT WITH CHRONIC HEMOLYTIC JAUNDICE

Three granule red cells and two reticulated red cells (basophilic) are shown. Compared to normal (fig. 1) the number of intact cells is greatly increased and fewer fragments are evident. Drawn like figure 1.

were hemolyzed; when 0.85 per cent was used fewer cells were hemolyzed. This indicates that cells already fragile to hypotonic salt solution tend to be destroyed by the combined effect of the solution and heat. The combined effect of 0.7 per cent salt solution and heat on normal cells resulted in the destruction of more cells, than when heat was allowed to act upon them in their own plasma or in 0.85

per cent salt solution, but the effect upon normal cells was not nearly so marked as the effect upon the patient's red cells treated in the same manner.

It is well recognized that with the increased red cell destruction, and constant renewal of these corpuscles in the circulation in chronic hemolytic jaundice, the blood is filled with varying, but increased numbers of recognizable young cells. The life of the mature red cell

TABLE 2

Comparison of specimens of normal blood with blood from two cases of chronic hemolytic jaundice, showing the effect of heat

	Normal (control)	Chronic hemolytic jaundice
Red blood cell count (millions per cubic millimeter).....	(A) 4.8 (B) 5.1	(A) 2.50 (B) 3.98
"Fragility" to hypotonic salt solution:		
Hemolysis began (per cent).....	(A) 0.40 (B) 0.42	(A) 0.54 (B) 0.60
Hemolysis complete (per cent).....	(A) 0.30 (B) 0.34	(A) 0.36 (B) 0.36
Per cent of red cells showing basophilia, reticulation or granules before heating in plasma.....	(A) 3.0 (B) 3.3	(A) 21.0 (B) 17.0
Per cent of intact red cells showing basophilia, reticulation or granules after heating in plasma.....	(A) 38.5 (B) 60.0	(A) 39.1 (B) 56.8
Per cent of intact red cells (after heating) of original total (estimated).....	(A) 10.0 (B) 10.0	(A) 60.0 (B) 50.0
Average number of intact red cells per oil immersion field after heating in plasma.....	(A) 9.9 (B) 14.6	(A) 238.0 (B) 60.0

in this disease is probably shorter than normal, because of the increased destruction of corpuscles. There thus should appear in the blood in this condition, a relatively larger number of *young mature* corpuscles, and comparatively few old ones, the latter presumably being the cells first removed by hemolysis. As young red cells are more resistant to heat than mature cells, those adult cells which resemble the immature ones in their heat resisting ability, may well

be the youngest of the mature cells. These are the cells which are so much more plentiful in the blood of chronic hemolytic jaundice than normal and which may occur in the blood of other pathologic conditions though never in such profusion. Differences in the character and rate of blood destruction and maturation and delivery of red cells can account for varying numbers of heat resistant adult erythrocytes in the circulation.

SUMMARY

1. The red blood corpuscles of normal human blood, when heated to 55°C. for one-half hour, undergo a profound and characteristic modification, with the production of fragmented forms, "shadows," microcytes, poikilocytes and a uniform distribution of the hemoglobin throughout the cell.

2. Immature erythrocytes—reticulocytes, polychromatophilic cells and granule red cells—of both normal and pathological blood are broken up less readily when heated to 55°C. than mature erythrocytes. The reticulated megalocytes are apparently an exception.

3. The difference between the effect of heat on the red cells of normal and pathological blood is not qualitative; it is quantitative, proportional to the number of immature cells.

4. There are two kinds of red cells which show no histological evidences of immaturity, and are classed as mature corpuscles. One kind resists the action of heating to 55°C. while the other is broken up and altered. The former represents a majority of the red blood corpuscles in chronic hemolytic jaundice. They are probably the younger of the mature cells.

BIBLIOGRAPHY

- Beale, L. S. *Quarterly Jour. Microscop. Sci.*, 1864, iv, 32. Observations upon the Nature of the Red Blood Corpuscles.
- Isaacs, R. *Arch. Int. Med.*, 1924, xxxiii, 197. Properties of Young Erythrocytes in Relation to Agglutination and Their Behavior in Hemorrhage and Transfusion.
- Klebs. *Centrl. f. d. med. Wissenschaften* 1863, i, 852. Die Formveränderung der rothen Blutkörperchen bei Laugethieren.
- Key, J. A. *Arch. Int. Med.*, 1921, xxviii, 511. Studies on Erythrocytes, with Special Reference to Reticulum, Polychromatophilia and Mitochondria.

- Krehl, L., and Marchand, F. Leipzig, 1908, i, 55. Handbuch der allgemeinen Pathologie.
- Rollett, A. New York, 1872, chapt. xiii, 263. A Manual of Histology—Stricker, S. et al. Am. Trans. edited by Buck, A. H.
- Schultze, M. Arch. f. mikro. Anat, 1865, i, 26. Ein heizbarer Objecttisch und seine verwendung bei Untersuchung des Blutes.